LIFE HISTORY, TERMINATION OF SUMMER DIAPAUSE, AND OTHER SEASONAL ADAPTATIONS OF *AGABUS DISINTEGRATUS* (CROTCH) (COLEOPTERA: DYTISCIDAE) IN THE CENTRAL VALLEY OF CALIFORNIA

R. Garcia
K.S. Hagen
W.G. Voigt

Division of Biological Control
University of California
Berkeley, CA 94720
U.S.A.

Quaestiones Entomologicae
26: 139–149 1990

ABSTRACT

The predaceous diving beetle, Agabus disintegratus, *breeds in temporary water sources, and adults pass the summer dry period in a state of diapause. Diapausing beetles bury themselves at the base of wetland plants until the pond is reflooded. Diapause is terminated in the laboratory by a short photoperiod, and weight changes and reproductive activity of field collected adults indicate that termination of diapause begins in the early fall before the ponds refill with water. Laboratory experiments indicate that microhabitats must maintain 100% relative humidity for long term survial of aestivating adults. Beetles could not be induced to fly during diapause. Flight activity corresponds to the most optimal period for finding natural water sources in California's Mediterranean climate.*

INTRODUCTION

Adults of the predaceous diving beetle, *Agabus disintegratus* (Crotch), pass the dry summer months in diapause (Garcia and Hagen 1985, Garcia and Hagen 1987). *A. disintegratus* is a univoltine species. Larval development takes place through the winter, and in early March teneral adults emerge, feed, and synthesize fat reserves through the spring prior to summer aestivation. As the water source dries, adults bury into the root layer and debris of plants in the basin of the pond, where they pass the summer. Although Young (1960) reported that *A. disintegratus* flew from drying ponds in Indiana, Garcia and Hagen (1987) could find no evidence for flight in adult *A. disintegratus* collected in the summer, even though the wings appeared to be normal.

The conditions and habitats in which *A. disintegratus* aestivates were described in Garcia and Hagen (1987). However, little is known about the adaptations of this beetle to the absence of water, its dispersal ability, or about conditions which regulate the termination of diapause. This report investigates these aspects of the biology of this aquatic beetle.

MATERIALS AND METHODS

Study Site.— Field observations and collections of *A. disintegratus* were made at approximately one month intervals from June 1986 through August 1988. All experimental materials were collected from pond #78 at the Gray Lodge

Wildlife Area, Butte County, California, which has been described in detail by Garcia and Hagen (1987). The basin of this pond is now almost completely covered with bermudagrass (*Cynodon dactylon* L.), in contrast to only partial coverage in 1986. Pond #78 was filled with water during the first week of October 1986, and remained full until the pond was drained in mid-May 1987. The pond was flood-irrigated for 3-6 days in June and again in July, 1987. In 1987, pond #78 was filled in early October and remained so until it dried in mid-April 1988. There were no summer irrigations in 1988.

Immatures.— Immature beetles were collected with a fine-mesh aquatic sweep net. Mature larvae, pupae and post-eclosion adults of *A. disintegratus* were collected from the soil on the north-western embankment above the water line of pond #78. A 5 cm diameter soil corer was used to sample the first 6 cm of soil, which was later sorted in the laboratory. These samples were taken at approximately 2 week intervals from March 2, 1988 through May 5, 1988, at which time no pupae or adults remained. Fifteen mature larvae were removed from their pupal cells, weighed and transferred to 2 cc vials with moist cotton on the bottom. The resulting pupae were reweighed within 1 day of pupation. Larval instar determinations were based on body length and head capsule width.

Adults.— Adult beetles were collected either from aquatic sweep net samples when standing water was present (October through April), or from the sod beneath the bermudagrass (late April through late September) (see Garcia and Hagen 1987). Beetles collected in the summer were sieved from bermudagrass sod collected with a shovel as described in Garcia and Hagen (1987). Other adults were reared in the laboratory from larvae and pupae collected from soil samples taken along the exposed banks of the pond.

Adult *A. disintegratus* collected from each field visit were weighed to ± 0.1 mg on an analytical balance and 5-10 specimens were dissected to determine reproductive activity and fat development as described by Garcia and Hagen (1987). Adults collected from water were blotted lightly with dry tissue paper before weighing. Beetles were weighed within 24 hrs after field collection or laboratory emergence. Teneral adult weights were determined by weighing 33 individuals within 1 day of eclosion. Beetles were held alive for up to one week until they could be dissected.

Weight Gain.— The rate of weight increase of adults was determined in the laboratory using newly emerged *A. disintegratus* adults. One to three-day old adults were placed individually in a 250 cc jars of water with a small piece of *Elodea* sp. for a resting substrate. The beetles were fed 20 late instar *Culex pipiens* L. larvae every other day and weighed at 7-10 day intervals until their weights stabilized.

Weight Loss.— Adult *A. disintegratus* in diapause were subjected to various levels of water moisture to estimate their ability to withstand desiccation. Eighteen adults were collected from sod samples on July 29, 1988, and weighed the following day. All beetles were held overnight (18 hrs) in 200 cc of water to equalize hydration levels and then reweighed . Half of the beetles (5 females, 4 males) were placed in 250 cc jars with moistened sand (100 g sand with 20 cc water), while the other group (5 females, 4 males) was placed in sand which had previously been dried to constant weight. Both groups were held at room temperature (20 ± 5°C). Beetles were weighed at 1–2 day intervals until no survivors remained in the dry sand group.

Weight loss of adult beetles at various relative humidities was measured as follows: relative humidities estimated at 100%, 90%, 69%, and 50% were attained by mixing 0, 10, 37.5, and 62.5 g of potassium hydroxide, respectively with 100 cc water in 0.94 l jars (Peterson 1959). Beetles were placed in refugia made from tubes of black cotton fabric (15 x 6 mm diameter) and closed at one end. The refugia provided a dark, constricted resting place which most beetles voluntarily entered. Two beetles (1 male & 1 female) in separate refugia were placed in a plastic cup (3 cm diameter), which was supported above the solution by a 2.8 cm diameter PVC pipe which had several holes drilled in it to facilitate equilibration of water vapor. A lid with 1.2 mm mesh screen was placed on the cups to prevent beetles from escaping. All jars were covered with a lid and placed in a sealed box to exclude light, and the box was then placed in a constant temperature cabinet at 21.5 ± 1°C. The beetles were weighed at 4-6 day intervals for 25 days. Body weights were transformed to proportion of original body weight at day 0. Regression lines based on these values over time were tested for parallelism, and *post hoc* comparisons of the slopes of the regression lines were made using Scheffé's multiple comparison (Marascuilo & Levin 1983).

Flight activity.— To determine time of year when *A. disintegratus* individuals could be induced to fly, field collected adult beetles were tested for flight within 2-3 days after collection and laboratory reared animals within 3 days of emergence. A 250 cc jar with a mouth opening of 6 cm in diameter was filled with 5 cm of sand and 150 cc of water. A round wooden stick about 10 cm in length and 0.3 cm in diameter was inserted vertically in the sand so that its apex extended about 1 cm above and in the center of the mouth of the jar. Ten jars were then placed in a water bath.

An individual beetle was placed into each jar and allowed to acclimate for at least 15 minutes before observations were started. Flight tests were usually conducted in full sun at mid-day, and run until water temperatures exceeded 30°C unless otherwise noted. Flight was determined from direct observations or from the presence of beetles in the surrounding water bath. The beetles were unable to escape the jar without flight; therefore any beetles which were missing or found in the water bath were assumed to have flown.

A single flight test was conducted on an overcast day (March 31, 1987) in which two randomly selected groups of 12 adults each (6 males, 6 females) were placed in water artificially heated from 23 to 36°C or in water kept at ambient temperatures (23 to 25°C).

Occasionally, adults were placed directly on loose dry soil exposed to full sun, and gross behavioral reactions recorded. Beetles observed to fly from any of the above conditions were recaptured when possible and dissected to determine reproductive condition and fat reserve.

Termination of diapause.— Adults used in this experiment (three groups of 5 pairs each) were collected from sod samples in May and June 1987. On June 29, each male/female pair was held in moistened sand (100 g sand and 15 cc water) in a 250 cc jar. A lid was placed on each jar to reduce evaporation. A 2 mm hole in the lid allowed for gas exchange. Each group of 5 jars was placed in one of three environmental chambers under the following conditions: "short light" (12L:12D hr) at 13°C and 18°C and "long light" (16L:8D) at 15°C. All temperatures were controlled to within ± 1°C. On September 9, 1987, the 14 surviving pairs from the three groups were placed in jars with 200 cc of tap water and one or two pieces of *Elodea* as an oviposition substrate under 16L:8D, 15°C conditions. Ten to 20 *Culex pipiens* L. larvae (3rd-4th instar) were given to the beetles every one or two days, and the plants were inspected daily for eggs from September 10 to September 18, 1987, at which time the beetles were dissected and examined for reproductive condition.

Another group of adults collected from sod on June 22, 1987 was paired by sex and placed directly in jars with 250 cc of water and one or two pieces of *Elodea* on June 25, 1987, and given 10 late instar *Cx. pipiens* larvae every three to five days. Three groups of five male/female pairs were placed under the same light regimes as the groups placed in moist sand. The *Elodea* was examined each week for eggs until the beetles were dissected September 2-4, 1987.

RESULTS

Immatures.— First instar *A. disintegratus* larvae were observed from October 26 through April 16, indicating continuous egg laying through the winter and early spring. Second instars were observed from November 23 through April 16, and third instars were observed from January 14 through April 16.

Mature larvae were observed leaving the pond to pupate from January 23 through April 12, 1988. Larvae crawled up the slope (a 1.5 m levee) to a vertical height of about 1 m above the water line and buried about 1-2 cm deep into the moist soil of the slope. Once under the soil, the larva shaped a spherical mud pupation cell about 6 mm in diameter. Several mature larvae but no pupae were observed inside their cells on February 17, 1988. Pupae were first observed on the following visit (March 2), and the first adults were recovered from pupal cells on March 15. In 1986 and 1987, teneral adults were first observed in the water on March 19, and March 26, respectively, but in 1988, they were not seen until April 12.

Mature larvae collected from the field inside their pupal cells weighed 23.1 ± 2.0 mg S.D. and the resulting pupae weighed 28.8 ± 1.8 mg (N = 15). The apparent increase in weight must have resulted from absorption of water, as they were held in a 100% humidity chamber on moist cotton.

Adults.— Figure 1 shows seasonal changes in adult body weight and fat reserves from eclosion to termination of diapause in the fall. A sharp increase in body weight and fat reserves was noted in the spring. Body weight and fat reserves remained relatively constant until mid-August when a decline was noted in both categories. After the pond was filled in the fall, body weights increased, but fat reserves declined.

No evidence of reproductive activity was noted in dissected adults collected from sod samples in the summer. Reproductive activity was observed in beetles collected from water in early October 1987, only five days after the field had been filled with water. Of five females dissected, all contained sperm in their spermathecae and 10-19 large developing eggs. Evidence of reproductive activity (*i.e.*, mature eggs in the ovarioles, sperm in the spermathecae, *etc.*) was observed from October through April.

In March and April of 1986 and 1987, both teneral and older generation adults were found together in the pond. In 1988, however, no older generation adults were seen in the pond after mid February. On February 15, 1987 more than a hundred adults (approx. 30 were collected within 30 m^2) were observed under plant debris along the levee of the pond. These were all reproductively active, older generation adults, as determined by dissection. On March 2, adults were not found on the bank, and could not be found in the pond by sweeping with an aquatic net. Only a small percent of the mature larvae in the soil samples had pupated by March 2, and teneral adults were not observed in pupal chambers until March 15. All adults had emerged from pupal cells and returned to the water by the end of April.

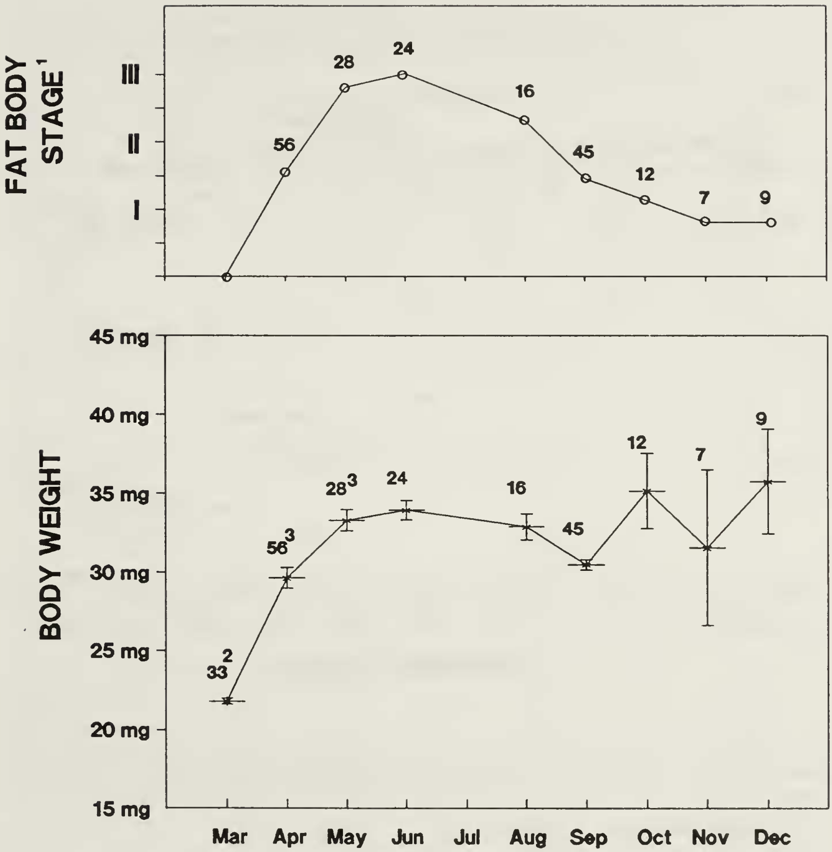

Fig. 1. Seasonal changes in average body weights and fat body reserves of field collected *Agabus disintegratus* Crotch from 1986-1988[1]. Midline horizontal line indicates mean. Vertical bars of weights indicate ± 2 standard error of the mean. The numbers indicate the number of beetles measured.

1 Fatbody stages: I, layer of fat bound to integument; II, free fat bodies; III, "filled" with free fat bodies.

2 Values for March represent weights from teneral adults reared from field collected pupae. Adults were weighed within 2 days of eclosion.

3 Only non-reproductive adults were included in the April and May averages, since they represented the current generation.

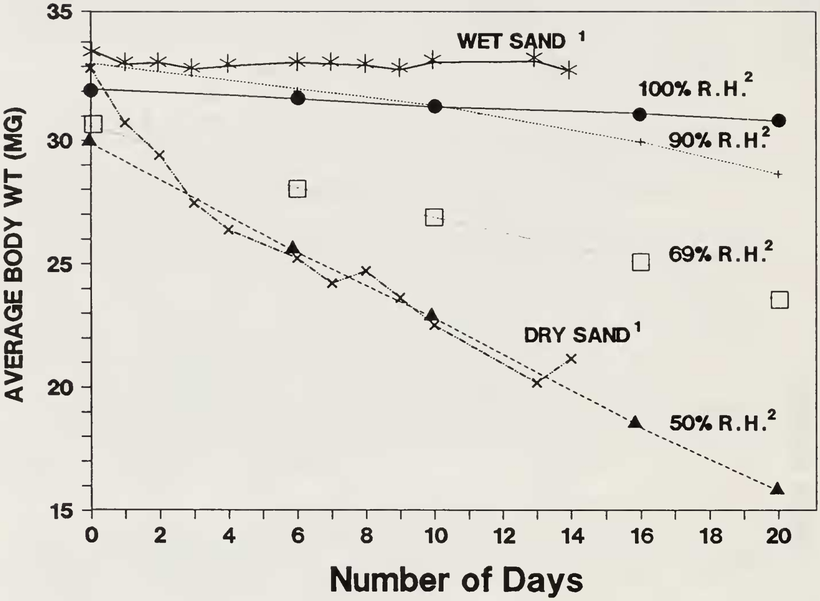

Fig. 2. Body weights of aestivating *Agabus disintegratus* Crotch under various conditions and relative humidities. The groups at various relative humidities were held in the dark in a 21 ± 1°C chamber.

[1] Held under room conditions (15-25°C).

[2] Held in dark at 21±1°C.

Table 1. *Post hoc* comparisons of slopes of weight loss vs. time for adult *A. disintegratus* under various conditions. Body weights were transformed to proportions of original body weights. Comparisons were of the slopes of regression lines using Scheffé's multiple comparison at P = 0.05.

	Test Conditions					
	Wet Sand	100%	90%	69%	50%	Dry Sand
Wet Sand	—	ns	sig	sig	sig	sig
100%		—	sig	sig	sig	sig
90%			—	sig	sig	sig
69%				—	sig	sig
50%					—	ns

Table 2. Tests for flight behavior and reproductive activity of *Agabus disintegratus*.

	Tested		Flew		Reproductive Activity[1]		
Date	Male	Female	Male	Female	Male	Female	Temperature Range (°C)
Jan-88	5	5	1	1	1A	1A	24–29
Feb-88	6	5	2	0	2A	—	30–35
Mar-87[2]	6	6	2	2	—	—	22–36
Mar-87[3]	6	6	0	0	—	—	22–25
Mar-88[4]	5	5	0	0	—	—	22–25
Mar-88[5]	2	2	2	0	2I	—	DRY SOIL[5]
Apr-87	32	32	13	11	8I 2A	4I 3A	22–36
May-87	10	10	0	0	—	—	29–32
Jun-87	10	10	0	0	—	—	27–34
Aug-87	5	5	0	0-	—	—	25–30
Oct-87	6	6	0	0	—	—	—
Oct-87	3	3	3	1	3A	1A	DRY SOIL[5]
Nov-87	5	5	2	2	2A	2A	21–26

[1] I = Reproductively inactive, A = Reproductively active. From beetles which were recovered after flight.

[2] In artificially heated water bath under overcast skies.

[3] Unheated water bath under overcast skies. Conducted concurrently with heated group.

[4] Reared from mature larvae collected February 2, 1988.

[5] Tested in shallow soil exposed to bright sun.

Table 3. Effects of photoperiod and substrate on reproductive conditions of field collected female *Agabus disintegratus*.

Substrate	Photoperiod (Light:Dark)	Temp. (°C)	N	Number Oocytes[1]	Number Mated[2]
Soil[3]	12L:12D	13.0	4	4	0
	12L:12D	18.0	2	2	2
	16L:8D	15.5	4	0	0
Water[4]	12L:12D	13.0	4	3	0
	12L:12D	18.0	2	2	1
	16L:8D	15.5	4	0	0

[1] Oocytes conspicuous in ovarioles at 10X

[2] Sperm present in spermathecae

[3] Held in soil from 29 Jun through 4 Sept, 1987, then placed in water and fed on mosquito larvae under 16L:8D photoperiod until dissected on 18 Sept.

[4] Held in water with mosquito larvae from 25 Jun until dissected on 2-4 September.

Weight Gain.— Teneral females averaged 21.3 ± 2.8 mg (N=20) and males averaged 22.4 ± 3.0 mg (N=13). Newly emerged adults fed 10 late instar mosquito larvae/day gained an average of 48% of their body weight in the first 10 days of observation.

Weight Loss.— Beetles placed in dry sand were found consistently on the surface, whereas those under moist sand remained buried. Results of the weight loss experiments are summarized in Figure 2. Body weights of the 100% R.H. and moist sand groups remained constant, while the beetles exposed to dry sand or lower humidities lost weight. The 50% R.H. group lost weight at about the same rate as the dry sand group, and rate of weight loss was inversely proportional to relative humidity. Regardless of the conditions of the experiment, all beetles that died had an average body weight of 19.8 ± 2.4 mg. There were no significant differences between the 100% R.H. and wet sand groups, nor between the dry sand and 50% R.H. groups. Otherwise, all other groups had significantly different slopes (Table 1).

Flight activity.— Flight from the test containers was observed in the laboratory during the fall, winter, and spring (Table 2). Beetles which flew during the flight tests typically climbed to the tip of the vertical stick, oriented with its back to the sun for up to 15 minutes, and then initiated flight. Some of the newly emerged, laboratory reared beetles were observed to attempt flight in indoor containers covered with clear plastic. This occurred in early March, after several days under dry but cool conditions (20–24°C). Beetles exposed to the sun on bare soil (March & October) attempted to fly sooner (*i.e.* in less than 15 min.) than beetles in water filled jars. In October 1987, 4 out of 6 beetles flew from soil, and 2 out of 4 beetles flew from soil in March 1988. No beetles were observed to fly from the surface of the water in any of the flight tests.

In the March 1987 test under overcast skies, four beetles flew from the heated water bath (at >31°C), while none of the beetles in the unheated water (maximum

22°C) attempted flight although three were observed to move up the stick and out of the water. All the beetles in the heated group moved up and down the stick or swam about their container as the water temperatures rose above 28°C. After water temperatures exceeded 32°C, the beetles spent the majority of time above the water on the stick. The unheated group usually remained below the surface grasping the stick near the sand except for occasional surfacing to breathe, but seldom climbed on the stick.

In April 1987, both reproductive (2 males, 3 females) and teneral adults (8 males, 4 females) flew. None of the beetles collected from summer sod samples were observed to fly, in spite of being exposed to high water temperatures.

Termination of Diapause.— Females collected in June and held with a male in either soil or water under a photoperiod of 12L:12D for 10 weeks exhibited reproductive activity upon dissection as indicated by conspicuous oocyte development and the presence of sperm in the spermathecae (Table 3). No eggs were observed on the *Elodea* in the holding containers under any of the conditions tested. Beetles under 12L:12D at both 13 and 18°C consumed 33.0 $\pm$ 4.2 S.D. and 33.2 $\pm$ 5.6 mosquito larvae, respectively, while those held at 16L:8D (15°C) consumed 28.6 $\pm$ 6.4 larvae. Differences in number of larvae consumed were not significant (Student's t-test, $P > 0.05$).

DISCUSSION

In the Central Valley of California, *A. disintegratus* is adapted to breeding and developing during the fall and winter months. Artificial inundation of pond 78 has occurred near the first week of October for the last two years, and adults have been observed mating by mid-October. In addition, dissection of adult females a few days after flooding in early October revealed developing oocytes and sperm in the spermathecae. Thus, reproductive activity begins in the early fall in these artificially flooded ponds. Under natural conditions, however, standing water, such as temporary ponds and vernal pools formed from precipitation and runoff, usually does not accumulate in the Central Valley until late November or December. Thus artificial flooding at the refuge allows for earlier development for *A. disintegratus*. Whether this is advantageous or not is unclear at this time, because despite observations of early reproductive development and the appearance of 1st instar larvae in late October, third instar larvae have not been seen until January. Since water temperatures are relatively mild through the fall, the occurrence of 3rd instar larvae should be expected much earlier in the season. We have no evidence at this time whether mortality or some physiological factor in larval development accounts for the lack of later instars.

The artificially manipulated water systems in waterfowl management areas may be an advantage for this species by providing more optimal conditions for adults during summer diapause, particularly in bermudagrass. Body weights of beetles collected through the dry summer remained relatively constant, which is apparently related to high humidities in the sod microhabitat. Maintenance of body weight through the summer may be partly influenced by the summer irrigations of the field. Summer irrigations not only provide standing water for a short period but more importantly, remoisten the rhizome debris layer where the beetles reside. In addition, growth and transpiration by the plants moderate the high temperature extremes that are common during the summer months in this part of California. Total rainfall for Colusa (15 km SW from the study site) during June, July, August and September, averaged 3.1, 1.0, 1.3, and 3.8 cm, (U.S. Weather Bureau summaries, 1980-1985). During 1987, however, no precipitation was recorded for the entire summer. Such small amounts of summer precipitation are normally insufficient to moisten the soil, especially under a thick canopy of bermudagrass. Consequently, moisture levels where these beetles reside would be dependent mostly on water retained in the soil from the spring or from water added by summer irrigations.

In the laboratory, adults placed in moist conditions maintained a constant body weight, but under totally dry conditions lost weight rather quickly and died within 15 days. This indicates that the microhabitat where the beetles reside cannot become completely dry even for relatively short periods during aestivation.

Natural habitats with wetland macrophytes such as cattails (*Typha* spp.) and bulrushes (*Scirpus* spp.), from which *A. disintegratus* has been recovered (Garcia and Hagen 1987), may provide a more suitable microhabitat than artificial habitats with bermudagrass. The soil around these more robust plants more readily forms cracks at the base of the stems which would allow beetles access into the deeper zones of the soil-root interface.

Beetles were able to fly throughout the fall, winter and spring, which corresponds to periods when standing water is likely to be available. During the summer, beetles did not fly, even after exposure to harsh conditions. The increased activity of beetles subjected to dehydration indicates that although beetles are unable to fly from drying conditions, they probably do crawl about in the field seeking more favorable microhabitats. This is highly adaptive in that other natural water sources are likely to be unavailable, and flight dispersal at that time would deplete fat and water reserves with little possibility of achieving success. By seeking out relatively insulated refugia for diapause, *A. disintegratus* avoids the extreme heat and dryness of the Central Valley in summer.

Female *A. disintegratus* dissected after being held in the laboratory with males in either soil or water under a photoperiod of 12L:12D were reproductively active, whereas the females held at 16L:8D were not, suggesting that diapause is terminated by a short-day photoperiod. Termination of summer diapause by short photoperiod has been demonstrated in several other insect species, including four different orders (see reviews in Tauber *et al*, 1986; Brown and Hodek, 1980). The decline in average body weight and fat reserves in late summer prior to inundation of the fields further suggests that termination of diapause begins while beetles are still in the dry state.

Several species of dytiscids exploit more permanent water sources, and through aestivation, *A. disintegratus* avoids competing with these species. In turn, *A. disintegratus* is able to exploit immediately temporary aquatic habitats as soon as they become available, without having to expend energy to seek them out through dispersal. Seasonal flight activity allows dispersal of reproductive and teneral adults during periods when natural water sources are more likely to occur.

ACKNOWLEDGEMENTS

We thank Kim Des Rochers and Aaron Goldberg for laboratory assistance for some of the experiments. R.B. Reno and Lee Ashford of the California Department of Fish and Game at the Gray Lodge Waterfowl Area provided logistical assistance. This study was supported in part by special funds for mosquito research in California.

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